DIFFERENT PREFERENCE REACTIONS OF THREE LAKE BAIKAL ENDEMIC AMPHIPODS TO TEMPERATURE AND OXYGEN ARE CORRELATED WITH SYMBIOTIC LIFE

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ABSTRACT

Thermopreference reactions and avoidance sensitivity to dissolved oxygen levels were measured in three endemic amphipod species from Lake Baikal: Eulimnogammarus verrucosus and E. vittatus, as well as Brandtia parasitica, which is a symbiont of the Baikalean sponge, Lubomirskia baikalensis. The benthic E. vittatus and E. verrucosus demonstrated highly developed thermopreference reactions, whereas B. parasitica did not show thermopreference behaviour. The experimental results also showed that E. vittatus and E. verrucosus avoid water with decreased dissolved oxygen levels, whereas behavioural reactions of B. parasitica did not depend on oxygen concentration. In the presence of sponge fragments, individual B. parasitica moved actively towards them, and concentrated on those fragments. Possible reasons for reduction of behavioural preference in B. parasitica are discussed with a focus on the species’ symbiotic status.

RÉSUMÉ

Les réactions de préférences à la température et la sensibilité d’évitement par rapport aux niveaux d’oxygène dissous ont été mesurées chez trois espèces d’amphipodes endémiques du lac Baïkal: Eulimnogammarus verrucosus et E. vittatus, ainsi que Brandtia parasitica, qui est un symbiose de l’éponge endémique Lubomirskia baikalensis. Les espèces benthiques E. vittatus et E. verrucosus ont démontré des réactions de préférences à la température hautement développées, tandis que B. parasitica n’en montrait pas. Les résultats expérimentaux ont aussi montré que E. vittatus et E. verrucosus évitaient l’eau ayant des niveaux bas d’oxygène dissous, tandis que les réactions comportementales de B. parasitica ne dépendaient pas de la concentration en oxygène. En présence de fragments d’éponge, les individus de B. parasitica se déplaçaient activement vers eux, et se concentraient sur ces fragments. Les possibles raisons de la réduction des préférences comportementales chez B. parasitica sont discutées avec une attention particulière au statut symbiotique de l’espèce.
INTRODUCTION

Aquatic animals live in environmental conditions that usually have daily and seasonal fluctuations. During their lifespan, aquatic animals can face various changes in their habitat conditions, some of which can be stressful. Behavioural reactions, such as avoidance or preference, constitute the first stress-responses of most animals. Temperature and oxygen are the main environmental factors that influence aquatic organisms. Thus, most species have developed a well-expressed behavioural adaptation, helping them to avoid harmful levels of abiotic factors and to select the optimal conditions available. Different preference and avoidance reactions are well known to many aquatic inhabitants, including crustaceans (Lindstrom & Fortelius, 1990; Lagerspetz, 2000; Lagerspetz & Vainio, 2006). However, in cases of closely related symbiont species, we can expect specific effects in their main preference reactions. It is well known that a symbiotic lifestyle frequently leads to behavioural changes.

The aim of this study was to establish the possible differences between a narrowly adapted symbiotic species and two free-living aquatic species, by their behavioural ability to select environmental conditions of temperature and oxygen.

METHODS

Freshwater Amphipoda were taken as experimental organisms for this study. This group is especially well represented in Lake Baikal, where more than 250 endemic species occur, inhabiting all depths of the lake (Takhteev, 2000). Behavioural preference reactions were studied in three endemic Baikalean amphipods: the free-living *Eulimnogammarus verrucosus* (Gerstfeld, 1858) and *E. vittatus* (Dybowski, 1874), as well as *Brandtia (Spinacanthus) parasitica* (Dybowski, 1874), a symbiont of the Baikalean sponge, *Lubomirskia baikalensis* (Dybowski, 1874).

*B. parasitica* with an average body size of 5 ± 1 mm, *E. verrucosus* with 30 ± 3 mm, and *E. vittatus* with 17 ± 1 mm were used in the experiments. Samples were collected in the summer months (2003-2004) at 0.5-20 m depth using dragnets and also by SCUBA diving, from Lake Baikal’s shoreline close to Irkutsk State University’s biological station at the settlement Bolshie Koty (southern Baikal). For species identification, the keys of Bazikalova (1945) were used.

As all the animals were taken from the littoral, which should have some daily fluctuation in conditions before the start of the experiments, the amphipods were pre-acclimated for laboratory conditions. Thus, all amphipods were kept for 2 days prior to the experiments in aerated 3-l tanks without food at 6-7°C. This helped us to avoid any possible effects of the degree of feeding, oxygen, and temperature that
might modify the amphipods’ behavioural activity (Lindstrom & Fortelius, 1992, 2001). To avoid possible behavioural or physiological modifications of the presence of unfamiliar substrates, known for some amphipods (cf. Chapelle & Peck, 1995), we used adequate substrates for all species: for both *Eulimnogammarus* sp., there were small stones or pebbles (20-30 mm), while for *B. parasitica*, next to stone, fragments of *L. baikalensis* were used as well. Only actively moving amphipods were used for the experiments.

The techniques for the thermopreference experiments followed Tsurnamal (1978) and Taylor (1984), with modifications. A device (size 2.5 × 0.3 m) (fig. 1A)

Fig. 1. Gradient installations used for the experiments: A, temperature gradient device; B, oxygen gradient device. For further explanations, see text.
was employed to create and maintain a water temperature gradient from 3°C up to 22°C. In this, the water formed a 5-6 cm deep trough, and a stable temperature gradient was established. The device was separated into 10 connected chambers or zones, each with their own temperature according to the gradient. Every chamber had a separate aeration tube and substrate (small stones or sponge’s fragments); the oxygen levels in the chambers were measured during the period when the temperature gradient was established. In most of the experiments, oxygen levels were close to maximal saturation (80-90%) at the selected temperature condition of each zone.

Temperature conditions were permanently measured with standard thermometers during all experiments and in all zones. At the start of the experiment, 20-30 amphipods were introduced into the installation. Every experiment included only one species to be tested. After a 1 hour acclimation period, the amphipods’ distribution was recorded every 30 min. for 5-6 hours. Usually, after the first 30 min. the individuals became distributed in a certain order, forming accumulations within the temperature zones preferred by that particular species. The figures present the average amphipod distribution as calculated during the whole experiment.

To define amphipod sensitivity towards a decrease in dissolved oxygen level, flowing water installations (0.25 × 0.60 m) with two parallel chambers were used (fig. 1B). In every chamber, a substrate (small stones or sponge’s fragments) was placed. Separate chambers were maintained for water with normal (11-12 mg O₂/l) and with lowered levels of oxygen. We decreased the dissolved oxygen level in the water by pumping air through it with a vacuum pump. The chambers were connected and the flows of water mixed. In each replicate, 20 amphipods were placed. Monitoring of the dissolved oxygen level in each chamber was realized during all experiments with Clark oxygen electrodes connected to an Oxymeter (I-130).

Experiments were carried out at 6-7°C. After a 1 hour acclimation period, amphipod distributions were observed every 30 min., for a total duration of 3-4 hours. Usually, after the first 30 min., individuals became distributed in a certain chamber preferred by that species. The figures present the average distribution of amphipods during the whole experiment.

We controlled the initial and subsequent levels of oxygen in the water. In the majority of experiments, the difference between initial and subsequent oxygen levels did not exceed 1-2 mg O₂/l (10-15% from initial); which was considered insignificant.

For each species, a series of at least 10 experiments was carried out, in different periods and with varying duration. All data were analysed statistically using Statistica 5.0 software (StatSoft). Analysis of variance (ANOVA) was used for comparison of the values obtained. In all figures, values are presented as
means ± standard deviation. Stars indicate significant differences from the controls at $p < 0.05$.

RESULTS

The results show different degrees of thermopreference behaviour. In fig. 2A, it is clear that *Eulimnogammarus verrucosus* prefers a temperature zone of 5-6°C, in which up to 30% of the specimens concentrated.

The distribution of *E. vittatus* (fig. 2B) comes close to that of *E. verrucosus*, with a maximal percentage found at temperatures of 5-6°C as well. However, a stronger thermopreference is evident for this species than for *E. verrucosus*, as in temperature zones higher then 10°C *E. vittatus* specimens were observed only occasionally.

Essentially different from the behaviour of the other amphipods studied, was the behaviour of the symbiotic *Brandtia parasitica* (fig. 2C). This species did not show any thermopreference behaviour in the gradient installations, as the individuals remained throughout the experiment in the zones where they had been placed initially. The lack of preference behaviour towards temperature in this species did not depend on the presence of any of the substrates used (stones or sponge fragments). Thus, we concluded that there is no thermopreference in this species.

Fig. 3A, B, C indicates the amphipods’ sensitivity to decreasing dissolved oxygen levels. *E. vittatus* and *E. verrucosus* largely avoided water with oxygen levels below 9-8 mg/l, and at a level of 7 mg/l, avoidance became close to 100%. On the other hand, the distribution of *B. parasitica* did not depend on oxygen concentration. The lack of avoidance-preference behaviour to oxygen in this species did not depend on the presence or absence of any form of substrate (stones or sponge fragments), either.

We noted, however, a different and distinct behavioural reaction that characterized *B. parasitica*: when we changed the substrate in both installations from small stones to fragments of sponge of the same size (or added sponge fragments to stones), individuals of *B. parasitica* actively moved towards those and concentrated around the sponge. This behavioural pattern was observed independently from the distance of the sponge fragments (up to 25 mm) and other conditional characteristics (i.e., temperature, oxygen level). Both *Eulimnogammarus* species, in contrast, used stones and sponge fragments indifferentially, i.e., without any visible preference.

DISCUSSION

The behavioural reactions found in the experiments should be discussed with a focus on the species’ ecological characteristics. *Brandtia parasitica* has a spatial
distribution that includes the entire shoreline of Lake Baikal, from 1.5 to 60 m depth, rarely down to 200 m. Its presence is strongly correlated with that of the Baikalean freshwater sponge, *Lubomirskia baikalensis* which it inhabits. Some researchers believe it is parasitic, feeding on the sponge’s body tissues (Kamaltynov et al., 1993; Dembitsky et al., 1994), others think it is merely commensal with
Fig. 3. Distribution of amphipods at different levels of dissolved oxygen, in %: normal oxygen level, 12-11 mg O₂ · l⁻¹; decreased oxygen levels: 1, 9-8 mg O₂ · l⁻¹; 2, 7-6 mg O₂ · l⁻¹; 3, less than 5 mg O₂ · l⁻¹. A, *Eulimnogammarus verrucosus* (Gerstfeld, 1858); B, *Eulimnogammarus vittatus* (Dybowski, 1874); C, *Brandtia parasitica* (Dybowski, 1874).

The other two amphipods have distributional characteristics close to that of *B. parasitica*. *Eulimnogammarus verrucosus* and *E. vittatus* are also widely distributed along Lake Baikal, occurring from several cm depth to 10-15 m (maximum 30 m). Both species prefer stony substrates. Thus, the littoral zone is the preferred habitat for all three species.

All species studied are known to have seasonal migration activity. Kamaltynov et al. (1993) described downward migrations of adult *B. parasitica* in the autumn,
at a time when the winds increase. They believed the migrations serve as a mechanism to avoid wave action. Vertical migrations from the shore to greater depths at the beginning of summer characterize *E. vittatus* and *E. verrucosus*. Avoidance of the increased water temperatures in the littoral in summer was discussed as a possible reason for the migrations of these species.

Despite the similarity in habitat, significant ecological differences between these three species exist: *E. verrucosus* and *E. vittatus* are active, benthic amphipods, whereas *B. parasitica* does not occur in the absence of its symbiont sponge. Living in the littoral with fluctuating conditions, both *Eulimnogammarus* species had to develop behavioural adaptations for selecting preferred, and avoiding harmful conditions. Thus, the preference and avoidance reactions in these species are well expressed. In contrast, the narrow specialization of *B. parasitica* has apparently resulted in the reduction of such behavioural reactions. The particular mechanisms of such a reduction still remain unclear and we can only speculate about them. So, in case of oxygen preference, reduction may be related to conditions of symbiotic life on the sponge that may provide constant oxygen levels. The apparent lack of thermopreference in *Brandtia parasitica* can also be linked to its close symbiotic association, in that it may not be able to readily move between sponges, on which it apparently depends so much, in a reaction to changes in lake conditions.

It should also be mentioned that the reaction we observed in active *Eulimnogammarus* species not only relates to their activity status, but also shows some differences that probably are related to their body size. As previously reported, the maximal amphipod size is dictated by the available environmental oxygen (Chapelle & Peck, 1999). Converting our data to the published graphs on linking the maximum size of amphipods with the oxygen concentration, as presented by Chapelle & Peck (2004), one can make the following observation: the *E. verrucosus* used in the experiments had an average size of 30 mm (which characterizes it as an adult). On the graph of Chapelle & Peck (2004) they should feel comfortable in water with at least 260 $\mu$mol O$_2$/kg, thus 8.3 mg/l. In our experiments we observe that more than 75% of the *E. verrucosus* individuals preferred this condition, and only 5% tolerated a lower (7-6 mg/l) concentration. *E. vittatus* used in the experiments had an average size of 17 mm (also characterizing it as an adult). On the graph presented by Chapelle & Peck (2004) they should feel comfortable in water with a lower oxygen concentration: at least 220 $\mu$mol O$_2$/kg, thus 7 mg/l. In our experiments, we observed a decreased sensitivity to avoid lower oxygen as compare to the larger-sized *E. verrucosus*. Such observation also confirms the possible ecological reasons for developing preference reactions in both *Eulimnogammarus* species, which have no significance for the symbiotic *B. parasitica*. On the other hand, *B. parasitica* has evidently developed specific reactions allowing the selection of the sponge, *Lubomirskia baikalensis*.
It should be noted that the parameters studied could not describe all possible particularities of the exact relationship with L. baikalensis. The current study is a first step to characterize the complex mutual interrelationship between these symbionts. The (lack of) behavioural reactions of Brandtia parasitica to its environment and the possible changes in its parameters remain an open question, to be answered by future studies.

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REFERENCES


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